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Lampiran 1. Ethical Clearance

KOMISI ETIK PENELITIAN KESEHATAN (KEPK)
FAKULTAS KEDOKTERAN UNIVERSITAS DIPONEGORO
DAN RSUP dr KARIADI SEMARANG
Sekretariat : Kantor Dekanat FK Undip Lt.3
Jl. Dr. Soetomo 18. Semarang
Telp/Fax. 024-8318350

RSUP Dr. KARIADI

ETHICAL CLEARANCE
No. 200/EC/FK-RSDK/2015

Komisi Etik Penelitian Kesehatan Fakultas Kedokteran Universitas Diponegoro-RSUP. Dr. Kariadi Semarang, setelah membaca dan meneliti Usulan Penelitian dengan judul :

GAMBARAN GEN JAK2 PADA PENDERITA POLISITEMIA VERA DI LABORATORIUM CEBIOR

Peneliti Utama : ***Kamelia Damaris Sihombing***

Pembimbing : 1. Prof. dr. Sultana M.H. Faradz, PhD
2. dr. Fanti Saktini, M.Si.Med

Penelitian : Dilaksanakan di Laboratorium CEBIOR
Fakultas Kedokteran Universitas Diponegoro Semarang

Setuju untuk dilaksanakan, dengan memperhatikan prinsip-prinsip yang dinyatakan dalam Deklarasi Helsinki 1975, yang diamended di Seoul 2008 dan Pedoman Nasional Etik Penelitian Kesehatan (PNEPK) Departemen Kesehatan RI 2011

Penelitian ini adalah Rekam Medik, jadi tidak memerlukan Informed Consent
Peneliti diwajibkan menyerahkan :

- Laporan kemajuan penelitian (clinical trial)
- Laporan kejadian efek samping jika ada
- Laporan ke KEPK jika penelitian sudah selesai & dilampiri Abstrak Penelitian

Semarang, 23 JUN 2015

Komisi Etik Penelitian Kesehatan
Fakultas Kedokteran Undip-RS. Dr. Kariadi
Ketua,

Prof. Dr.dr. Suprihati, M.Sc, Sp.THT-KL(K)
NIP.19500621 197703 2 001

Lampiran 2. Pemeriksaan *Jak2V617F*

a. Ekstraksi DNA (Metode Salting Out)

Bahan : Darah vena dengan antikoagulan EDTA volume 5-10 cc

Reagen:

1. NH4Cl lysis buffer
2. Strong TE buffer
3. Normal TE buffer
4. SDS 10% (Sodium Deodecyl Sulfat)
5. Proteinase K Enzim 10mg/mL
6. NaCl 6M
7. Ethanol 70%
8. Ethanol Absolute

Cara Kerja:

1. Memasukkan 5-10cc darah ke dalam tabung 15mL + NH4Cl lysis buffer add 15 mL.
   Membiarkan darah hemolisis 10-30 menit dalam suhu 4ºC

   Centrifuge 3500 RPM selama 10 menit
   Membuang supernatant, pellet di resuspensi sempurna dengan jentikan jari
   Melaakukan seperti diatas hingga mendapatkan pellet putih
2. Pelet putih + 2cc strong TE buffer

   + 50µL PK (30 saja sudah cukup)
   + 100 µL SDS 10%

   Mencampur hingga sempurna, memasukkan dalam waterbath 50% selama semalam.

b. Proses ARMS PCR

1. Mengencerkan hasil ekstraksi DNA hingga knsentrasi kira-kira 100 nano (untuk mengukur konsentrasi DNA, gunakan nano drop)

2. Membuat master mix untuk PCR (50µL), 2µL DNA ditambah master mix 48 µL, memasukkan dalam mesin PCR.

Pada tahap ini akan terjadi proses:

-	Initial denaturation	suhu 95º	selama 5 menit
-	Denaturation	suhu 94º	selama 1 menit
-	Annealing	suhu 75º	selama 45 detik
-	Extension	suhu 57º	selama 30 detik
-	Final extension	suhu 4/20º	selama 5 detik

Kelima tahap diatas dikerjakan selama 35 siklus hingga didapatkan PCR product/produk PCR.

c. Elektroforesis pada gel dan interpretasi

1. Pembuatan gel agarose 1%

- Melarutkan serbuk agarose 0,50 gr dalam 50mL buffer TBE satu kali
- Memanaskan dengan mencampur terlebih dahulu dengan ethidium bromide
- Mencetak dan menunggu sampai membeku
- Menambahkan loading die (pemberat dan pewarna) ke produk PCR kemudian memasukkan ke dalam gel

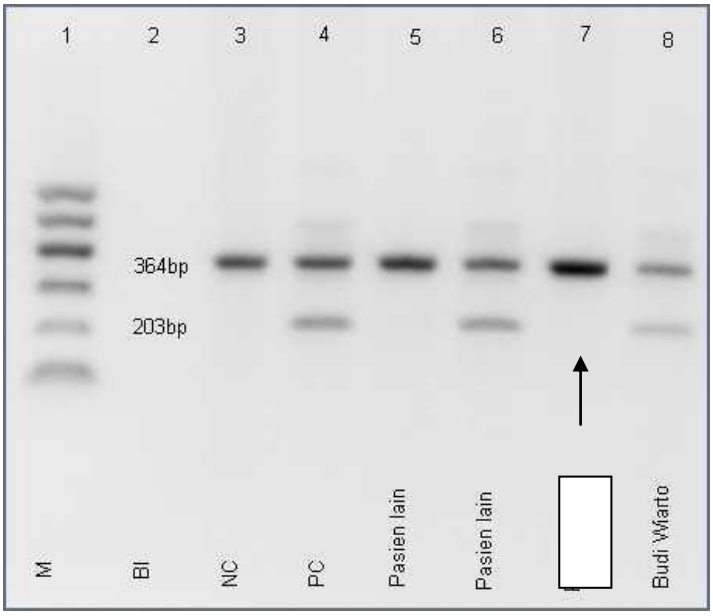
2. Melarikan (running) gel elektroforesis dalam bak elektroforesis dengan voltage 120V selama 30 menit.
3. Interpretasi hasil dibaca di gel doc.

   Jika kontrol negative maka hanya terdapat satu band (364bp/basepaired). Jika kontrol positif maka terdapat 2 band di 203 bp (sebagai mutand band) dan 364 bp.

d. Interpretasi hasil

Hasil Pemeriksaan Analisis Mutasi JAK2 V617F

Pemeriksaan yang dikerjakan : ARMS-PCR JAK2 V617F



→(tanda panah): Pasien

Lajur 1 kontrol Marker 100 bp, lajur 2 blank kontrol, lajur 3 kontrol negatif , lajur 4 kontrol positif dengan mutasi gene JAK 2 V617F didapatkan dua fragmen dengan ukuran 364 bp dan 203 bp, lajur 5,6 dan 7 pasien lain, lajur 8 hasil amplifikasi DNA pasien didapatkan dua fragmen maka didapatkan mutasi gene JAK 2 V617 F. Pemeriksaan ini merupakan pemeriksaan kualitatif yang tidak membedakan keadaan homozigot/heterozigot.

Kesimpulan : Ditemukan mutasi gen JAK2 V617F pada pasien ini.

Lampiran 3. Dokumentasi Praktikum

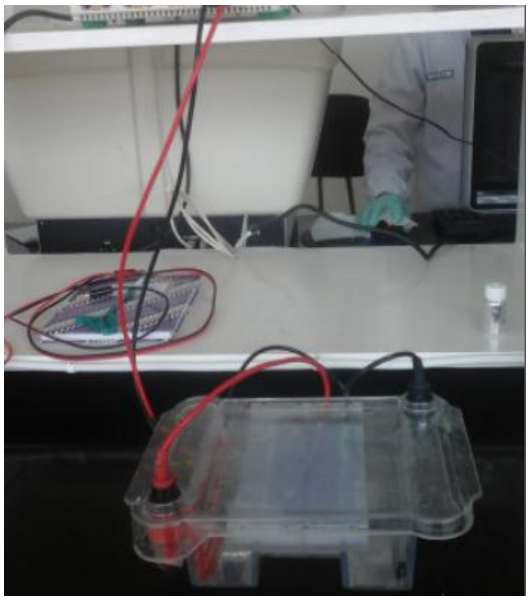
Ekstraksi DNA

Waterbath

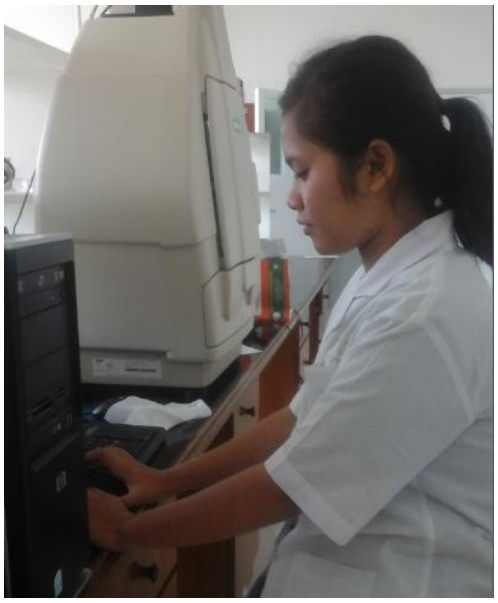
Sentrifugasi

PCR

Gel Elektroforesis

Pembacaan di gel document



Lampiran 4. Biodata Mahasiswa

BIODATA MAHASISWA

Nama	: Kamelia Damaris Sihombing
NIM	: 22010111120054
Tempat/tanggal lahir	: Lintongnihuta 21 Maret 1992
Jenis kelamin	: Perempuan
Alamat	: Jl. Gondang Timur 1 no. 45B
Nomor telepon	: 082323589430
e-mail	: kameliasihombing@gmail.com

Riwayat Pendidikan Formal

1.	SD	: St. Yoseph Bintang Kejora	Lulus tahun: 2005
2.	SMP	: SMP N 1 Lintongnihuta	Lulus tahun: 2008
3.	SMA	: SMA N 2 Soposurung Balige	Lulus tahun: 2010
4.	FK UNDIP	: Masuk tahun:2011	